



Evaluating robustness of a diesel-degrading bacterial consortium isolated from contaminated soil

Sydow, Mateusz; Owsianiak, Mikolaj; Szczepaniak, Zuzanna; Framski, Grzegorz; Smets, Barth F.; awniczak, ukasz; Lisiecki, Piotr; Szulc, Alicja; Cyplik, Pawe; Chrzanowski, ukasz

Published in:
New Biotechnology

Link to article, DOI:
[10.1016/j.nbt.2016.08.003](https://doi.org/10.1016/j.nbt.2016.08.003)

Publication date:
2016

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Sydow, M., Owsianiak, M., Szczepaniak, Z., Framski, G., Smets, B. F., awniczak, ., Lisiecki, P., Szulc, A., Cyplik, P., & Chrzanowski, . (2016). Evaluating robustness of a diesel-degrading bacterial consortium isolated from contaminated soil. *New Biotechnology*, 33(6), 852-859. <https://doi.org/10.1016/j.nbt.2016.08.003>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Citation:**

2 Sydow, M., Owsianiak, M., Szczepaniak, Z., Framski, G., Smets, B.F., Ławniczak, Ł., Lisiecki, P.,
3 Szulc, A., Cyplik, P., Chrzanowski, Ł., 2016. Evaluating robustness of a diesel-degrading
4 bacterial consortium isolated from contaminated soil. N. Biotechnol. 33, 852–859.
5 doi:<http://dx.doi.org/10.1016/j.nbt.2016.08.003>

Evaluating structural robustness of a diesel-degrading bacterial consortium isolated from contaminated soil

Mateusz Sydow^{*,a}

Mikołaj Owsianiak^b

Zuzanna Szczepaniak^c

Grzegorz Framski^d

Barth F. Smets^e

Łukasz Ławniczak^a

Piotr Lisiecki^a

Alicja Szulc^a

Paweł Cyplik^f

Łukasz Chrzanowski^a

^a Institute of Chemical Technology and Engineering, Poznan University of Technology, Berdychowo 4, 60-965 Poznań, Poland

^b Division for Quantitative Sustainability Assessment, Department of Management Engineering, Technical University of Denmark, Produktionstorvet, Building 424, DK-2800 Kgs. Lyngby, Denmark

^c Institute of Food Technology of Plant Origin, Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland

^d Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland

^e Department of Environmental Engineering, Technical University of Denmark, Miljøvej, Building 113, DK-2800 Kgs. Lyngby, Denmark

^f Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Wojska Polskiego 48, 60-627 Poznań, Poland

* corresponding author

Tel.: + 48 61 6653716

Fax.: + 48 61 6653649

e-mail: mateusz.sydow@gmail.com (M. Sydow)

Abstract

It is unknown whether diesel-degrading bacterial communities are structurally and functionally robust when exposed to different hydrocarbon types. Here, we exposed a diesel-degrading consortium to model either alkanes, cycloalkanes, or aromatic hydrocarbons as carbon sources to study its structural resistance. The structural resistance of the consortium was low, with changes in relative abundances of up to four orders of magnitude, depending on hydrocarbon type and bacterial taxon. The low resistance is explained by the presence of hydrocarbon-degrading specialists in the consortium and differences in the growth kinetics on individual hydrocarbons. However, despite this low resistance, the structural and functional resilience were high, as verified by re-exposing the hydrocarbon-perturbed consortium to diesel fuel. The high resilience is either due to the short exposure time, insufficient for permanent changes in consortium structure and function, or the ability of some consortium members to be maintained during exposure on degradation intermediates produced by other members. In summary, the consortium is expected to cope with short term exposures to narrow carbon feeds while maintaining its structural and functional integrity, which remains an advantage over biodegradation approaches using single species cultures.

Keywords

biodegradation, community dynamics, hydrocarbon, robustness, resilience

1. Introduction

Selection of microbial communities for bioaugmentation of soils contaminated with hydrocarbon mixtures, such as diesel fuel, must consider their ability to adapt to temporal changes in hydrocarbon composition over the course of biodegradation [1, 2]. Similarly, if bioremediation relies on the activity of autochthonous microorganisms, temporal changes in the community structure and function can occur [3–5]. The ability of microbial communities to resist to such, potentially irreversible, changes is one of the factors determining the success of bioremediation [6]. This ability, often referred to as robustness, is usually characterized by investigating: (i) the ability of a community to resist a change in its structure after perturbation; and (ii) the potential for recovery of the community's structure to its initial state after removal of the perturbation. These two indicators of structural robustness are referred to with the terms *structural* resistance and *structural* resilience, respectively [7, 8]. The structure of a community may also influence its *functional* resilience, understood as the ability of a community to maintain a particular activity despite perturbation [7, 9, 10].

Vila et al (2010) showed that successive biodegradation of particular hydrocarbon fractions in the marine environment is conducted by different, temporally dominant bacterial taxa [11]. Also Kostka et al. (2011) showed that *Alcanivorax* was the dominant taxon during linear and branched alkanes utilization in the early stages of crude oil biodegradation in marine environment, whereas *Acinetobacter*, *Marinobacter* and *Pseudomonas*, identified as both alkane and aromatics degraders, were the most abundant at the later stage of biodegradation [2]. Diesel-degrading consortia are similarly not thought to consist of generalist bacteria with ability for growth on all major

hydrocarbon types (that is, linear and branched alkanes, cycloalkanes and aromatic hydrocarbons) present in a petroleum diesel fuel [1]. It is expected that the structural resistance of diesel-degrading consortia is low. If a given hydrocarbon (e.g., the branched-chain alkane pristane) is utilized by one consortium member only, the change in structure of the consortium, when exposed to that hydrocarbon, will be governed by the initial fraction of cells belonging to that consortium member, the kinetics of growth of the degrading member on that hydrocarbon, and the exposure time to the hydrocarbon. On the other hand, if a hydrocarbon can be utilized by many members of the consortium, its change in structure will depend mainly on the differences in the kinetics of growth between consortium members on that hydrocarbon. Both exposure time and the kinetics of growth are expected to play a key role in determining the structural resilience: the ability of the perturbed community to recover its initial state.

Resistance and resilience of microbial communities must be considered when constructing consortia for bioaugmentation of hydrocarbon mixtures [6]. Low resistance is undesirable if a consortium isolated on a specific hydrocarbon mixture, e.g. petroleum diesel fuel, is exposed to various hydrocarbons present in the diesel fuel over the course of biodegradation, unless the consortium is structurally and functionally resilient. Ideally, the consortium should be able to cope with narrow carbon feeds and adapt readily to varying composition of a hydrocarbon mixture over time. To date, there is limited knowledge on the structural resistance and resilience of diesel-degrading bacterial consortia associated with exposure to different hydrocarbon types.

In this paper, we use a diesel-degrading bacterial consortium to evaluate its: (i) *structural resistance*, measured as the degree of change in structure in terms of abundance of the dominant bacterial taxa when deprived of its typical energy source (i.e., petroleum diesel fuel) and perturbed to grow on individual model aliphatic, cycloaliphatic, or aromatic hydrocarbons; (ii) *structural resilience*, measured as the degree of recovery of the perturbed consortium to its initial state when re-exposed to diesel fuel after its perturbation; and (iii) *functional resilience*, measured as the ability of the previously perturbed and re-exposed consortium to mineralize the diesel fuel. The relative abundance of seven core taxa, used to describe the structure of the perturbed and recovered communities, was quantified using real-time PCR and the ddCt method for relative quantification [12]. In total, 6 aliphatic (*n*-dodecane, *n*-hexadecane, *n*-octadecane, *n*-docosane, heptamethylnonane, pristane), 5 cycloaliphatic (decalin, cycloheptane, ethylcyclohexane, butylcyclohexane, bicyclohexyl), and 8 aromatic (acenaphthene, ethylbenzene, 1,5-dimethyltetraline, o-xylene, cyclohexylbenzene, naphthalene, 2-ethylnaphthalene, phenanthrene) hydrocarbons, which represent major hydrocarbon types present in petroleum diesel fuel, were employed. In addition, biodiesel (a mixture of fatty acid methyl esters) derived from rapeseed, was used. Thereby, we show how the overall low structural resistance depends on the type of hydrocarbon and bacterial taxon, and how the perturbed consortium recovers its initial state, presenting high structural and functional resilience.

2. Materials and methods

2.1. Bacterial consortium

120 The bacterial community employed in this study had been isolated from a soil contaminated with
121 crude oil using selective enrichments with diesel fuel as source of carbon and energy [13]. The
122 community contained bacteria of the following taxa: *Achromobacter* sp. (AchrP), *Alcaligenes* sp.
123 (AlcP), *Citrobacter* sp. (CKK), *Comamonadaceae* (ComP), *Sphingobacterium* sp. (SphiP),
124 *Pseudomonas* sp. (PseuP), and *Variovorax* sp. (VariP) [14]. The community has a degradation
125 potential toward diesel and biodiesel fuels [13–16], and is able to mineralize all the individual
126 hydrocarbons employed in this study [17].

127 The community was stored in 30% (v/v) glycerol stocks at -80°C. To prepare an inoculum, a
128 stock suspension (1 mL) was transferred to a 300 mL Erlenmeyer flask containing 50 mL of mineral
129 medium [13] and petroleum diesel fuel (0.5%, v/v), and was cultivated for 24 h at 25°C on an
130 orbital shaker (120 rpm). Then, a 1 mL aliquot of the cell suspension was transferred to a new
131 enrichment flask and the culture was grown for 3 days in the same conditions. This step was
132 repeated three times and cells from the last enrichment were centrifuged at 10,000×g, washed twice
133 with 40 mL of the mineral medium, re-suspended in the medium, and used as inoculum. This
134 inoculum is further referred to as the initial community.

135

136 2.2. Hydrocarbons

137 To study the structural resistance of the community against carbon source changes, a total of 19
138 individual hydrocarbons representing all major hydrocarbon types present in petroleum diesel fuel,
139 were used (Table 1). Structural resistance was also assessed against 4 model hydrocarbon mixtures
140 and against biodiesel derived from rapeseed (Table 1). The hydrocarbons were purchased from
141 Sigma Aldrich. The biodiesel, produced according to DIN E 51606 [18] was purchased from a
142 supplier in Germany, whereas petroleum diesel fuel, produced according to EN 590:2004 [19] was
143 purchased from a petrol station (PKN Orlen, Poland). Prior to experiments, all the fuels had been
144 sterilized by filtration (Millex, pore size of 0.2 µm, Millipore).

145

146 2.3. Repeated exposure to hydrocarbons

147 The experimental design is presented in Figure 1. The structural resistance of the community was
148 evaluated by comparing the relative abundances of core taxa within the initial community (that is,
149 the preculture grown on diesel fuel) with that of the communities perturbed by the growth on
150 individual or mixtures of defined hydrocarbons (Table 1) as sources of carbon in repeated growth
151 experiments. First, cells (1-mL cell suspension) were transferred from the inoculum culture to 500-
152 mL bottles containing 50 mL medium and hydrocarbon or hydrocarbon mixture (including diesel
153 fuel as initial carbon source) at concentrations given in Table 1 and cultivated at 25°C at 120 rpm
154 for 7 days. Then, aliquots were transferred to new set of bottles containing medium (the ratio of
155 inoculum volume to total liquid volume was 1:50) and same hydrocarbons at their respective
156 concentrations and cultivated in the same conditions for 7 days. This step was repeated 3 times,
157 reaching 5 steps in total.

158 The structural resilience of the community was evaluated by comparing the structure of the
159 initial community with that of the communities first perturbed by growth on individual
160 hydrocarbons or defined mixtures, as described above, and then returned to grow on complex
161 petroleum diesel fuel as sole carbon source. Again, growth on diesel was repeated by 2 sequential

dilution passages (again, the ratio of inoculum volume to total liquid volume was 1:50), reaching 3 steps in total. Aliquots from the last growth passage were sampled to determine community structure and are referred to as recovered communities.

To assess the functional community resilience, we compared the initial and recovered communities with respect to the mineralization kinetics of petroleum diesel fuel in saturated sand microcosms.

2.4. Structure of the community

Real-time PCR and the ddCt method for relative quantification (12), employed earlier by Cyplik et al. (2011) [14], were used to quantify the relative abundance of the core taxa, from which we described the structure of the perturbed and recovered communities. In the ddCt methods, the relative abundance is expressed as relative quantity (RQ), where the amount of target rRNA genes for the seven bacterial taxa retrieved from the studied communities is normalized to the total number of bacterial rRNA in the respective community (Eq. 1).

$$RQ = \frac{\left(\frac{q_{t(x)}^T}{q_{t(x)}^B} \right)}{\left(\frac{q_{t(0)}^T}{q_{t(0)}^B} \right)} = \frac{X_{N,q}}{X_{N,cb}} = (1 + E)^{-(\Delta C_{T,q} - \Delta C_{T,cb})} = (1 + E)^{-\Delta \Delta C_T} = 2^{-\Delta \Delta C_T} \quad \text{Eq. 1.}$$

where q_t^T is the quantity of a taxon of interest (i.e., target in ddCt terms) in any perturbed or recovered community (i.e., sample) at time t equal to 5 and 8 weeks for the perturbed and recovered communities, respectively; q_t^B is the quantity of total bacteria (i.e., reference) in the sample at time t ; q_0^T is the quantity of the target in the initial community (i.e. calibrator) at time t equal to zero; q_0^B is the quantity of the reference in the initial community at time t equal to zero. $X_{N,q}$ and $X_{N,cb}$ are thus the normalized amount of the target taxon in the sample and the normalized amount of the target taxon in the initial community, respectively. In the ddCt method, the RQ is computed from the difference in threshold cycles for the target and the reference in a sample ($\Delta C_{T,q}$) and the difference in threshold cycles for target and reference in a calibrator ($\Delta C_{T,cb}$). The efficiency of the target (E) was assumed equal to 1 [12].

Biomass was collected by centrifugation of the liquid culture at 8228×g for 15 min. Total DNA was extracted and purified using Genomic Mini kit (A&A Biotechnology, Poland) following the manufacturer's instruction with initial pretreatment with lysozyme (45 mg/mL), lysostaphin (200 U/mL) and mutanolysin (250 U/mL). The characteristics of primers and probe sets for the PCR can be found in Cyplik et al. (2011) [14].

2.5. Mineralization kinetics of diesel fuel in saturated sand microcosms

Mineralization of diesel fuel was studied in saturated sand microcosms, as described in Lisiecki et al. (2014) [20]. Briefly, 50 g of dry sand was placed in a sealed 1-litre glass bottles. The microcosms were spiked with diesel fuel (16 g/kg dry sand) applied on the sand surface. Then, the microcosms were inoculated with the initial community, or with the recovered community (re-

200 exposed to diesel fuel after exposure to hydrocarbons) by applying a dense cell suspension (1 mL;
201 OD_{600nm} 3 ± 0.1) on the sand surface. Afterwards, 14 mL of mineral medium was added to obtain full
202 saturation. Microcosms were maintained without disturbance at 20°C for 28 days. The
203 mineralization was determined by measuring CO₂ content in a base trap (10 mL of 0.75 M NaOH in
204 a 20 mL vial) placed in microcosms. Titration of the diluted NaOH and Na₂CO₃ solution from the
205 trap with 0.1 M HCl was done using an automatic titrator (Metrohm titroprocessor 686). Each
206 experiment was carried out in triplicates.

208 3. Results

209
210 The response of the studied consortium to model hydrocarbons was hydrocarbon- and taxon-
211 specific (Fig 2, left panel). Both increases and decreases in relative taxon abundance, up to four
212 orders of magnitude relative to the initial community, were observed.

213 When exposed to *n*-alkanes, the largest changes in abundance were found for *Citrobacter* sp.
214 (an increase of four orders of magnitude), and *Achromobacter* sp. (a decrease of three orders of
215 magnitude) (Fig 2a). The response for other community members was somewhat smaller, within
216 one order of magnitude. For branched-alkanes, the *Alcaligenes* sp., *Achromobacter* sp., *Citrobacter*
217 sp., *Comamonadaceae* and *Pseudomonas* sp. taxa increased in relative abundance up to three orders
218 of magnitude after exposure to heptamethylnonane and pristane (Fig 2a). On the other hand,
219 *Sphingobacterium* sp. decreased in relative abundance after exposure to branched alkanes,
220 especially pristane. No significant changes were observed for *Variovorax* sp. For cycloalkanes, an
221 increase in abundance of up to two orders of magnitude (*Achromobacter*, *Comamonadaceae* and
222 *Variovorax* sp.) was observed, while *Citrobacter* sp. and *Sphingobacterium* sp. did not significantly
223 change in their abundance (Fig. 2b). For aromatic hydrocarbons, *Alcaligenes* sp.,
224 *Comamonadaceae*, *Pseudomonas* sp., *Sphingobacterium* sp. and *Variovorax* sp. were, in most
225 cases, up to four orders of magnitude more abundant in comparison to their relative quantity in
226 initial community, whereas the abundance of *Achromobacter* sp. and *Citrobacter* sp. decreased up
227 to three orders of magnitude (Fig. 2c).

228 Overall, these results suggest that the structural resistance of the consortium was low.
229 However, when these hydrocarbon-perturbed cultures were re-exposed to diesel fuel, the relative
230 abundance of the dominant taxa returned close to the values in the initial community (Fig. 2e-h).
231 The RQ values (log₁₀-transformed) ranged from -0.5 to 0.5. Further, in the 28-day mineralization
232 kinetics test, all recovered communities showed similar kinetics of diesel mineralization (Fig. 2i-l).
233 This suggests that the ability to degrade diesel fuel, did not change, and functional resilience was
234 high.

236 4. Discussion

238 4.1. Explaining low structural resistance and high resilience

239 Structural changes in the community are expected when deprived of its normal energy source, the
240 diesel fuel, and forced to survive on a single hydrocarbon. Allison and Martiny (2008) already

showed that the composition of microbial communities is sensitive to changes in various carbon amendments, including petroleum [21]. Although biodegradation of individual hydrocarbons was not verified in the present study, the consortium did have a potential to mineralize all the studied hydrocarbons when supplied as a mixture [17], suggesting that each individual hydrocarbon was degraded by one or more community members also when supplied as sole source of carbon and energy. This is further confirmed by an increase in turbidity that was observed in the flasks due to cell growth.

Hydrocarbon toxicity is not likely to have influenced the community structure as the consortium had been adapted to relatively high (>5 mg/L) concentration of diesel fuel [13], and individual hydrocarbons were applied at subinhibitory levels. Thus, an increase of RQ values of a taxon when exposed to a specific hydrocarbon can indicate that either: (i) the hydrocarbon was a primary carbon and energy source for that taxon, or (ii) the hydrocarbon was not a primary carbon and energy source for that taxon but the taxon benefited from its biodegradation by another community member(s). On the other hand, a decrease in relative abundance could indicate that either: (i) a taxon did not have the ability to grow on the hydrocarbon and did not benefit from its biodegradation by other member(s), or (ii) the taxon degraded the hydrocarbon but its specific growth rate was smaller compared to other members of the consortium.

Allison and Martiny (2008) similarly showed that the composition of microbial communities does not recover for some time after disturbance [21]. Although we did not evaluate the functional resistance of the studied bacterial community during the study, a significant changes in mineralization of diesel fuel during stress (passages on single hydrocarbons) are expected to occur. The soil microbial community structure and functions can be both positively or negatively correlated depending on the used perturbation and measured function [22]. However, the mineralization of petroleum hydrocarbons (or hydrocarbon mixtures) seem to change with changing microbial community structure, since not all microorganisms present in the environmental communities can degrade all available carbon sources [1, 23]. Hamamura et al. (2013) already showed that mineralization of ^{14}C -hexadecane was different among the same soil samples with diverse microbial community structures (induced by the contamination of soil with different hydrocarbon mixtures) [5]. In our study, despite the apparently low structural resistance, the structural and functional resilience were relatively high. This may suggest that either: (i) each identified consortium member was able to grow on each studied hydrocarbon, albeit at various rates; or (ii) not each consortium member was able to grow on each studied hydrocarbon, but the exposure time was short enough to avoid irreversible changes in community structure. The latter explanation is more likely as in diesel-degrading consortia, it is known that some bacteria degrade a wide variety of hydrocarbons (and are therefore generalists), while others are specialized to few compounds (and are therefore specialists) [1].

4.2. Hydrocarbon-degrading specialist and generalists in the consortium

Specialists are likely found in taxa that displayed the highest difference in relative abundance between individual hydrocarbons, such as seen for some branched-chain alkane or aromatic hydrocarbon exposures.

282 Linear alkanes are generally easier to degrade as compared to the branched ones [24–27]. In
283 our consortium, however, *Sphingobacterium* sp. was the only alkane-degrading taxon that had
284 decreased RQ values when exposed to branched alkanes. By contrast, *Achromobacter* sp. increased
285 in relative abundance when exposed to branched alkanes only (when exposed to *n*-alkanes a
286 decrease in relative abundance was observed). Thus, *Sphingobacterium* sp. could be dominated by
287 strains which have alkane oxidation mechanisms specific to *n*-alkanes, such as β -oxidation, whereas
288 *Achromobacter* sp. could be dominated by bacteria which have alkane oxidation mechanisms
289 specific to branched alkanes, such as ω -oxidation [28]. Another example of specialized alkane-
290 degraders could be strains within the *Citrobacter* sp. taxon, which increased significantly in relative
291 abundance when exposed to *n*-alkanes or biodiesel, but less when exposed to branched alkanes and
292 cycloalkanes, and decreased in relative abundance when exposed to all aromatic hydrocarbons. This
293 is supported by the fact that *Citrobacter* sp. showed the highest increase in relative abundance
294 among all taxa when exposed to biodiesel, which was expected as biodegradation of fatty acid
295 methyl esters from biodiesel proceeds through the pathway known for *n*-alkanes (i.e. through fatty
296 acid intermediates [26]). This is also in agreement with the ability of *n*-alkane degraders to grow on
297 the *n*-alkane oxidation products [29].

298 Metabolic pathways of cycloalkanes are less characterized than those for linear or branched
299 alkanes [26, 30]. During oxidation of a cyclic alkanes dicarboxylic acids are usually formed,
300 similarly to ω -oxidation of branched alkanes [28, 30]. This could explain why the species expected
301 to be primary *n*-alkane degraders, such as *Citrobacter* sp. or *Sphingobacterium* sp. did not increase
302 in abundance when exposed to cyclic alkanes. By contrast, based on RQ values *Alcaligenes* sp.,
303 *Comamonadaceae* are expected to be dominated by generalists with regard to their potential for
304 degradation of alkanes, with both β -oxidation and ω -oxidation mechanisms co-occurring within
305 these taxa [31].

306 Apart from *Achromobacter* sp. and *Citrobacter* sp., all taxa increased in relative abundance
307 when exposed to aromatic hydrocarbons. This is consistent with the ability of AlcP, ComP, PseuP
308 SphiP and VariP to degrade various aromatic hydrocarbons [32–41]. Relatively large increases in
309 abundance after exposure to aromatic hydrocarbons are associated with somewhat lower increase in
310 abundance of the taxa when exposed to *n*-alkanes, indicating that aromatic hydrocarbons are the
311 preferential carbon source within the studied community. However, bacteria belonging to
312 *Pseudomonas* sp. and *Alcaligenes* sp. are known to degrade a wide variety of compounds, including
313 alkanes (e.g. dodecane, pristane) [37, 42, 43], cycloalkanes (e.g. cyclohexane, decalin) [37, 44, 45]
314 and aromatic hydrocarbons (e.g. benzene, phenanthrene) [35, 36, 37, 39, 40] and are thus expected
315 to be hydrocarbon-degrading generalists.

317 5. Conclusions

318
319 We showed that a diesel-degrading bacterial consortium was structurally and functionally robust
320 when employed for biodegradation of various hydrocarbons. The robustness of the microbial
321 community was evaluated by investigating the structural and functional resilience and resistance.
322 Despite low structural resistance, which was explained by the presence of hydrocarbon-degrading
323 specialists in the consortium and differences in the kinetics of growth, the structural and functional

324 resilience were high. The robustness of the diesel-degrading consortium is an advantage when
325 employed for biodegradation (e.g. bioaugmentation) of environments which may have varying
326 hydrocarbon composition over time. Such a consortium is expected to be able to cope with narrow
327 carbon feeds yet maintaining structural and functional integrity, which is advantageous over
328 biodegradation carried out by single species.

329 Our findings raise several additional questions. First, it is unknown whether the results are
330 applicable to other hydrocarbon-degrading consortia isolated on complex hydrocarbon mixtures.
331 Second, it is unknown whether the structural and functional robustness is a property of consortia
332 isolated from contaminated environments, or whether such a (robust) consortium can be constructed
333 from single species of known ability to degrade specific hydrocarbons. Third, the applicability of
334 these results to field conditions needs to be examined as mass transfer limitations of carbon sources
335 and availability of nutrients may play a large role in shaping community structure. Finally, it is
336 unknown whether the consortium maintains its structural and functional integrity if longer exposure
337 times are used. Biodegradation time scales in soils or aquifers are longer than a few weeks, in which
338 case structural robustness and functional performance might be challenged.

340 **Acknowledgements**

341
342 This research was funded by the National Science Centre of Poland in the framework of DEC-
343 2013/11/D/NZ9/00100 project (grant no. 2013/11/D/NZ9/00100). Dr. Ireneusz Miesiąc (PUT) is
344 gratefully acknowledged for supplying with the biodiesel fuel, and for providing access to analytical
345 instruments.

347 **References**

- 348
349 [1] Ciric L, Philip JC, Whiteley AS. Hydrocarbon utilization within a diesel-degrading bacterial
350 consortium. FEMS Microbiol Lett 2010;303:116–22.
351
352 [2] Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A et al. Hydrocarbon-
353 degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted
354 by the deepwater horizon oil spill. Appl Environ Microb 2011;77:7962–74.
355
356 [3] Ciric L, Griffiths RI, Philp JC, Whiteley AS. Field scale molecular analysis for the monitoring
357 of bacterial community structures during on-site diesel bioremediation. Bioresour Technol
358 2010;101:5235–41.
359
360 [4] Hamamura N, Olson SH, Ward DM, Inskeep WP. Microbial population dynamics associated
361 with crude-oil biodegradation in diverse soils. Appl Environ Microbiol 2006;72:6316–24.
362

363 [5] Hamamura N, Ward DM, Inskeep WP. Effects of petroleum mixture types on soil bacterial
364 population dynamics associated with the biodegradation of hydrocarbons in soil environments.
365 FEMS Microbiol Ecol 2013;85:168–78.
366

367 [6] Bell TH, Yergeau E, Juck DF, Whyte LG, Greer CW. Alteration of microbial community
368 structure affects diesel biodegradation in an Arctic soil. FEMS Microbiol Ecol 2013;85:51–61.
369

370 [7] Little AEF, Robinson CJ, Peterson SB, Raffa KF, Handelsman J. Rules of engagement:
371 interspecies interactions that regulate microbial communities. Annu Rev Microbiol 2008;62:375–
372 401.
373

374 [8] Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML, Young VB. Reproducible
375 community dynamics of the gastrointestinal microbiota following antibiotic perturbation. Infect
376 Immun 2009;77: 2367–75.
377

378 [9] Gentile ME, Nyman JL, Criddle CS. Correlation of patterns of denitrification instability in
379 replicated bioreactor communities with shifts in the relative abundance and the denitrification
380 patterns of specific populations. ISME J 2007;1:714728.
381

382 [10] Bell CW, Acosta-Martinez V, McIntyre NE, Cox S, Tissue DT, Zak JC. Linking microbial
383 community structure and function to seasonal differences in soil moisture and temperature in a
384 Chihuahuan Desert grassland. Microb Ecol 2009;58:827–42.
385

386 [11] Vila J, Nieto JM, Mertens J, Springael D, Grifoll M. Microbial community structure of a heavy
387 fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic
388 hydrocarbon utilization. FEMS Microbiol Ecol 2010;73:349–62.
389

390 [12] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time
391 quantitative PCR and the 2- $^{-\Delta\Delta CT}$ method. Methods 2001;25:402–08.
392

393 [13] Owsianiak M, Szulc A, Chrzanowski Ł, Cyplik P, Bogacki M, Olejnik-Schmidt AK et al.
394 Biodegradation and surfactant-mediated biodegradation of diesel fuel by 218 microbial consortia
395 are not correlated to cell surface hydrophobicity. Appl Microbiol Biotechnol 2009;84:545–53.
396

397 [14] Cyplik P, Schmidt M, Szulc A, Marecik R, Lisiecki P, Heipieper HJ et al. Relative quantitative
398 PCR to assess bacterial community dynamics during biodegradation of diesel and biodiesel fuels
399 under various aeration conditions. Bioresource Technol 2011;102:4347–52.
400

401 [15] Owsianiak M, Chrzanowski Ł, Szulc A, Staniewski J, Olszanowski A, Olejnik-Schmidt AK et
402 al. Biodegradation of diesel/biodiesel blends by a consortium of hydrocarbon degraders: Effect of
403 the type of blend and the addition of biosurfactants. Bioresource Technol 2009;100:1497–1500.
404

405 [16] Chrzanowski Ł, Dziadas M, Ławniczak Ł, Cyplik P, Białas W, Szulc A et al. Biodegradation
406 of rhamnolipids in liquid cultures: Effect of biosurfactant dissipation on diesel fuel/B20 blend
407 biodegradation efficiency and bacterial community composition. *Bioresource Technol*
408 2012;111:328–35.
409

410 [17] Lisiecki P. The influence of biodiesel on biodegradation of diesel fuel. PhD dissertation.
411 Poznan University of Technology 2013.
412

413 [18] DIN E 51606. Liquid fuels - Diesel fuel of fatty acid methylester (FAME) – Specifications,
414 German Institute for Standardization 1997.
415

416 [19] EN 590:2004. Automotive fuels - Diesel - Requirements and test methods, European
417 Committee for Standardization 2004.
418

419 [20] Lisiecki P, Chrzanowski Ł, Szulc A, Ławniczak Ł, Białas W, Dziadas M et al. Biodegradation
420 of diesel/biodiesel blends in saturated sand microcosms. *Fuel* 2014;116:321–27.
421

422 [21] Allison SD, Martiny JBH. Resistance, resilience, and redundancy in microbial communities. *P*
423 *Natl Acad Sci USA* 2009;105:11512–19.
424

425 [22] Riah-Anglet W, Trinsoutrot-Gattin I, Mertin-Laurent F, Laroche-Ajzenberg E, Norini M-P,
426 Latour X, et al. Soil microbial community structure and function relationships: A heat
427 stress experiment. *Appl Soil Ecol* 2015;86:121–30.
428

429 [23] Mariadassou M, Pichon S, Ebert D. Microbial ecosystems are dominated by specialist taxa.
430 *Ecol Lett* 2015;18:974–82.
431

432

433 [24] Olson JJ, Mills GL, Herbert BE, Morris PJ. Biodegradation rates of separated diesel
434 components. *Environ Toxicol Chem* 1999;18:2448–53.
435

436 [25] Rojo F. Degradation of alkanes by bacteria. *Environ Microbiol* 2009;11:2477–90.
437

438 [26] Rojo F. Enzymes for aerobic degradation of alkanes. In: Timmis K, editor. *Handbook of*
439 *hydrocarbon and lipid microbiology*, Berlin. Springer Berlin Heidelberg; 2010, p. 781–97.
440

441 [27] Parales RE. Hydrocarbon degradation by Betaproteobacteria. In: Timmis K, editor. *Handbook*
442 *of hydrocarbon and lipid microbiology*, Berlin. Springer Berlin Heidelberg; 2010, p. 1715–24.
443

444 [28] Solano-Serena F, Marchal R, Vandecasteele J-P. Biodegradation of aliphatic and alicyclic
445 hydrocarbons, In: Vandecasteele JP, editor. *Petroleum microbiology: concepts, environmental*
446 *implications, industrial applications*, Paris. Editions Technip; 2008, p. 186–211.

447
448 [29] Dashti N, Al-Awadhi H, Khanafer M, Abdelghany S, Radwan D. Potential of hexadecane-
449 utilizing soil-microorganisms for growth on hexadecanol, hexadecanal and hexadecanoic acid as
450 sole sources of carbon and energy. *Chemosphere* 2008;70:475–79.
451
452 [30] Kirkwood KM, Chernik P, Foght JM, Gray MR. Aerobic biotransformation of decalin
453 (decahydronaphthalene) by *Rhodococcus* spp.. *Biodegradation* 2008;19:785–94.
454
455 [31] Johnson RJ, West CE, Swaih AM, Folwell BD, Smith BE, Rowland SJ et al. Aerobic
456 biotransformation of alkyl branched aromatic alkanoic naphthenic acids via two different pathways
457 by a new isolate of *Mycobacterium*. *Environ Microbiol* 2012;14:872–82.
458
459 [32] Meyer S, Moser R, Neef A, Stahl U, Kampfer P. Differential detection of key enzymes of
460 polyaromatic-hydrocarbon-degrading bacteria using PCR and gene probes. *Microbiology*
461 1999;145:1731–41.
462
463 [33] Jeon CO, Park W, Ghiorse WC, Madsen EL. *Polaromonas naphthalenivorans* sp. nov., a
464 naphthalene-degrading bacterium from naphthalene-contaminated sediment. *Int J Syst Evol Micr*
465 2004;54, 93–97.
466
467 [34] Przybulewska K, Wieczorek A, Nowak A. Isolation of microorganisms capable of styrene
468 degradation. *Polish J of Environ Stud* 2006;15:777–83.
469
470 [35] Arun A, Raja PP, Arthi R, Ananthi M, Kumar KS, Eyini M. Polycyclic aromatic hydrocarbons
471 (pahs) biodegradation by basidiomycetes fungi, *Pseudomonas* isolate, and their cocultures:
472 Comparative *in vivo* and *in silico* approach. *Appl Biochem Biotechnol* 2008;151: 132–42.
473
474 [36] Farhadian M, Vachelard C, Duchez D, Larroche C. In situ bioremediation of monoaromatic
475 pollutants in groundwater: A review. *Bioresource Technol* 2007;99:5296–5308.
476
477 [37] Plaza GA, Lukasik K, Wypych J, Nałęcz-Jawecki G, Berry C, Brigmon RL. Biodegradation of
478 crude oil and distillation products by biosurfactant-producing bacteria. *Polish J of Environ Stud*
479 2008;17:87–94.
480
481 [38] Weelink SAB, Tan NCG, ten Broeke H, van den Kieboom C, van Doesburg W, Langenhoff
482 AAM et al. Isolation and characterization of *Alicyclophilus denitrificans* strain bc, which grows on
483 benzene with chlorate as the electron acceptor. *Appl Environ Microbiol* 2008;74:6672–81.
484
485 [39] Cao B, Nagarajan K, Loh K-C. Biodegradation of aromatic compounds: current status and
486 opportunities for biomolecular approaches. *Appl Microbiol Biotechnol* 2009;85:207–28.
487

- 488 [40] Deveryshetty J, Phale PS. Biodegradation of phenanthrene by *Alcaligenes* sp. strain PPH:
 489 partial purification and characterization of 1-hydroxy-2-naphthoic acid hydroxylase. FEMS
 490 Microbiol Lett 2010;311:93–101.
- 491
- 492 [41] Satola B, Wübbeler JH, Steinbüchel A. Metabolic characteristics of the species *Variovorax*
 493 *paradoxus*. Appl Microbiol Biotechnol 2013;97:541–60.
- 494
- 495 [42] Yuste L, Corbello ME, Turiégano MJ, Karlson U, Puyet A, Rojo F. Characterization of
 496 bacterial strains able to grow on high molecular mass residues from crude oil processing. FEMS
 497 Microbiol Ecol 2010;32:69–75.
- 498
- 499 [43] Rocha CA, Pedregosa AM, Laborda F. Biosurfactant-mediated biodegradation of straight and
 500 methyl-branched alkanes by *Pseudomonas aeruginosa* ATCC 55925. AMB Express 2011;1:9.
- 501
- 502 [44] Kumar M, Leon V, De Sisto Materano A, Ilzins OA, Galindo-Castro I, Fuenmayor SL.
 503 Polycyclic aromatic hydrocarbon degradation by biosurfactant-producing *Pseudomonas* sp. IR1. Z
 504 Naturforsch A 2006;61c:203–12.
- 505
- 506 [45] Anderson MS, Hall RA, Griffin M. Microbial metabolism of alicyclic hydrocarbons:
 507 Cyclohexane catabolism by a pure strain of *Pseudomonas* sp. J Gen Microbiol 1980;120:80–94.
- 508

509 **Figure captions**

- 510
- 511 Fig. 1. Experimental design for evaluating structural resistance and resilience and functional
 512 resilience of a diesel-degrading bacterial consortium.
- 513
- 514 Fig 2. Relative quantity (RQ) values (in log₁₀ scale) of hydrocarbon-perturbed cultures of the
 515 diesel-degrading bacterial consortium (a-d); of the hydrocarbon-perturbed cultures re-exposed to
 516 diesel fuel (e-h), and diesel fuel mineralization kinetics with respect to recovered communities and
 517 initial community (i-l). Error bars represent standard errors of the mean.

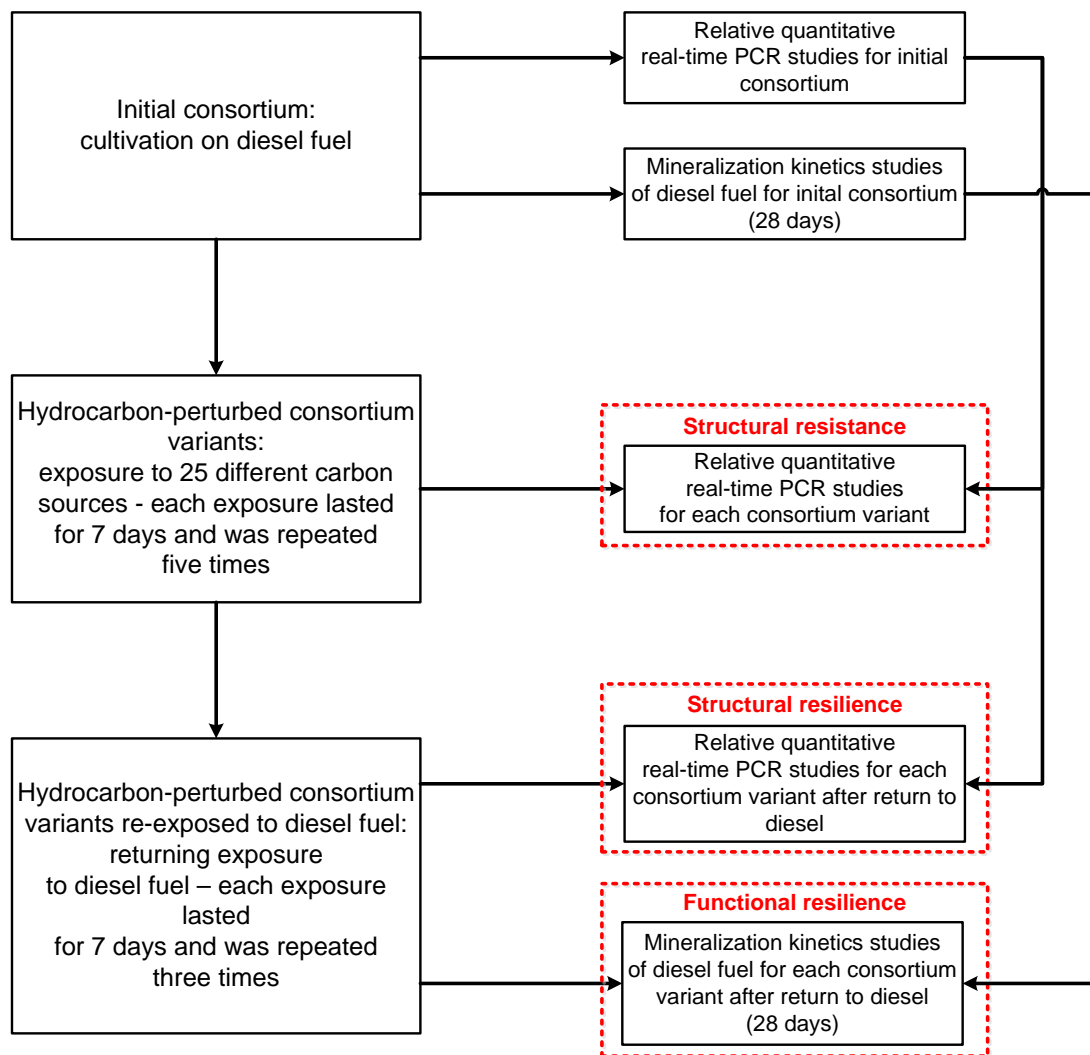


Fig. 1.

